

THE IMMUNE RESPONSE OF THE LABORATORY MOUSE  
SUBJECTED TO TRICHINELLA SPIRALIS AND  
PREDNISONE

An abstract of a Thesis by  
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The problem. This project studied the effect of prednisone on the immune system of young white laboratory mice infected with Trichinella spiralis.

Procedure. Mice were divided into the following four groups: 1) T. spiralis-infected, 2) prednisone-injected, 3) T. spiralis-infected and prednisone-injected, and 4) normal controls. Three days prior to the inoculation of T. spiralis to groups one and three, prednisone injections were initiated on alternate days to groups two and three. At the end of the thirty day life cycle of T. spiralis, sera was drawn from the four groups for an agar gel-diffusion and precipitin test to show prednisone's effect on the immunoglobulin production due to the Trichinella larvae. The number of larvae in the diaphragm muscle was also determined to insure infection had occurred, and to show prednisone's effect on the susceptibility to infection.

Findings. The "T. spiralis-infected:prednisone-injected" group had a 23% higher frequency of larvae present in the diaphragm muscle than did the "T. spiralis-infected" group. Prednisone treated mice demonstrated a marked decrease in weight gain as compared to the other groups.

Conclusions and Recommendations. Prednisone appeared to immunosuppress the mice, resulting in an enhanced Trichinella infection, and decreased the total weight gain of the mice. For further study, the use of multiple infections, a longer period of time before the sera is collected, and perhaps heavier infection doses of Trichinella may facilitate the antibody study. A histological study would be useful in investigation of the effects of prednisone on the immuno-competent regions.

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A Thesis  
Presented to  
The School of Graduate Studies  
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by  
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## INTRODUCTION AND REVIEW OF THE LITERATURE

Most warm-blooded carnivores of the cold and temperate climates are natural hosts of the nematode, Trichinella spiralis (Faust, 1970). Infection by T. spiralis occurs when a host eats muscle containing living encapsulated larvae (Gould, 1945; Kagan, 1960; and Larsh, 1963). The larvae are released from the cysts as the muscle surrounding them is digested by the gastric juices of the host. After the larvae excyst in the stomach or duodenum, they become embedded in the mucosal lining for 24 hours. A series of molts occur (the exact number is undetermined) which result in the development of mature adults in the intestine 24 to 48 hours after ingestion of the larvae.

The adult Trichinella male is 1.4 to 1.6 mm in length and the female is approximately 2.2 to 3.6 mm long. The male has two cone-shaped appendages that are utilized in copulation. The adult male and female Trichinella reinvade the mucosa and copulation occurs. The impregnated females are embedded deeper in the mucosa whereas the males are not and are shed by the host.

T. spiralis is ovoviviparous and living larvae are released by the gravid female beginning about day six post-infection. The larvae enter the lacteals, travel to the regional lymph nodes, and via the lymphatics enter the venous blood. The larvae pass through the heart-lung route, and into the peripheral blood capillaries where they burrow into

skeletal muscle. Larval production occurs for 15 to 18 days after infection before the adult female is expelled.

The Trichinella larvae show a predilection for well-oxygenated skeletal muscle (Gould; 1945; Hill, 1957; and Larsh, 1963). Once T. spiralis has penetrated the muscle, it becomes encapsulated to await recycling by another carnivore.

Larsh (1963) indicates that there are three pathogenic phases in the life cycle of Trichinella. The first is the gastro-intestinal stage. At four days post-infection, an inflammatory response occurs in the small intestine of the host, lasting 14 days, with the most acute inflammation occurring between days six and nine. During this phase, depending upon the degree of infection, the host may experience fever, diarrhea, anorexia, and weight loss.

The second pathogenic phase is the circulatory stage, resulting in antigen-antibody responses, vasculitis, hemorrhaging, inversion of lymphocyte and neutrophil ratios, eosinophilia, and a variety of other humoral and cell mediated responses (Markell and Voge, 1971).

The final pathogenic phase occurs in the skeletal muscle, resulting in myositis beginning at the 14th day post-infection. Neutrophils and macrophages attack the muscle fibers around the Trichinella larvae, and this attack is followed by the development of a hyaline wall from the thickened sarcolemma that surrounds the larvae, forming a

capsule. This capsule becomes invested by capillaries and will eventually calcify. Inflammatory cells in the area are replaced by plasma cells, representing sites of local antibody production (Catty, 1969).

T. spiralis has been studied more thoroughly than any other parasitic worm in relation to cell mediated immunity. Larsh and his co-workers (1964) confirmed the transfer of immunity with the use of peritoneal-exudate cells from infected donors. This conferred immunity with the peritoneal-exudate cells was probably unrelated to antibody production since Larsh was unable to detect antibodies in the recipients 21 days after the peritoneal-exudate cell transfer by the techniques of indirect hemagglutination, bentonite flocculation, latex agglutination, and indirect fluorescent antibody. No transfer of immunity was seen in cells given to mice from uninfected donors. Kim, et al., (1967) demonstrated the successful transfer of immunity in mice against the adult worm of this parasite by the use of lymphoid cells from donor mice infected with T. spiralis.

Larsh (1970) showed that splenic lymphocytes from mice artificially sensitized with the crude larva-extract antigen inhibited the migration of macrophages in capillary tubes. The demonstration of a migratory inhibition factor with cells from mice four days after infection was also confirmed. This indicates that a larval substance is the functional antigen in producing delayed hypersensitivity.



Larsh (1972) showed that neonatal thymectomy delayed the expulsion of adult Trichinella worms without interfering with the antibody production, and that antilymphocytic globulin prevented the usual degree of expulsion after a challenge dose. When both modes of treatments were given, a significant number of worms were still being harbored six weeks after infection. Larsh also showed that lymphoid cells from hyperinfected donors conferred immunity to the recipients, while large volumes of immunoglobulin did not.

DiNetta (1972) reported on the usefulness of antilymphocytic serum in experimental trichinosis. Mice infected with T. spiralis undergo a characteristic abrupt expulsion of adult worms from the gut during the second week of a primary infection. This "spontaneous cure", accepted as an immunological phenomenon, can be suppressed by corticosteroids and antilymphocytic serum. DiNetta showed that the administration of heterologous anti-mouse-lymphocyte serum to mice completely suppressed the "spontaneous cure". Treatment effectiveness was time dependent. When initiated two days prior to inoculation with T. spiralis, the anti-mouse-lymphocyte sera was successful, but when given one day after inoculation, it was ineffective.

Many serologic tests will detect antibodies during an infection with T. spiralis. Kagan and Norman (1970), Sadun (1972), and Crandall and Crandall (1972), have shown that immunocytes in the intestinal mucosa of infected mice contain

IgG, IgM, and IgA antibodies, and that the intestinal contents contain mostly IgA. Humoral factors were not believed to be important in the expulsion of adult worms from the small intestine, since serum antibody levels do not indicate the host's immune status (Gore, 1970), nor do serum antibodies transfer immunity by the use of anti-serum (Larsh, 1972).

Larsh (1963) speculated that the antibodies directly interfered with the metabolism of the adult Trichinella worms and caused the demonstrated stunting of growth and the reduction of the reproductive potential of the adults while still present in the intestine.

Between 1963 and 1972, many of Larsh's studies reported data which support the hypothesis that the immunity of mice against adult worms of T. spiralis has a cell mediated basis, which follows a humoral antibody interaction. A specific delayed hypersensitivity is produced in response to an allergin within, or released by, the mature larvae within the muscle during infection. With a challenge dose of living larvae, the delayed hypersensitivity now is thought to cause an allergic inflammation due to injury of cell and tissues of the small intestine. This results in biochemical changes in the intestine, and produces unfavorable conditions leading to expulsion of the worms. According to this concept, the specific delayed hypersensitivity reaction is the primary, but indirect event, which leads to

the expulsion. The resultant inflammatory changes in the tissues are secondary, but the direct event, which leads to the elimination of the worm.

Larsh (1972) showed evidence that antithymocyte and antilymphocyte injections retarded the expulsion of worms. However, these results seem more circumstantial for the mechanism of "cell mediated" expulsion of the adult worms. At the present time, the role of humoral factors is uncertain for this particular aspect of total immunity to T. spiralis.

Larsh (1974) reported results from his studies involving histopathological observations of mice that had received spleen cells from donors sensitized to T. spiralis and treated with antilymphocytic serum. The recipients of these spleen cells showed a much greater lymphoid response at two days post-infection than controls injected with spleen cells from normal mice. No transfer of humoral antibodies was detected by the indirect fluorescent antibody technique. The early presence of mononuclear cells, including lymphocytes, are characteristic of delayed hypersensitivity reactions, and were particularly significant in this experiment since the pool of cells from the suppressed donors contained some committed T-cells. The acute inflammation stage was also initiated earlier, with eosinophilis present in all tissues, and later, neutrophils. The early presence of these cells, along with lymphocytes, suggests an interaction

of T-cells with a specific tissue antigen, leading to the release of a chemotactic factor for the accumulation of the eosinophils. This factor is released in response to tissue injury produced by the adult Trichinella worms. A close association between the inflammatory response and the effectiveness of the expulsion mechanism supports the theory that after a cell mediated response, (subsequent to the humoral interaction), the inflammatory tissue changes are directly responsible for the elimination of the adult worms.

There has been much evidence about the close association between humoral and cellular factors and intestinal inflammation leading to the expulsion of the adult worms from the anterior half of the small intestine (Larsh and Weatherly, 1974). This paper focuses on the importance of humoral factors and their role in producing the demonstrated effect of the removal of the worms from the host.

A variety of antigens have been shown in the metabolic products (excretion and secretion antigens), somatic portion, and cuticle of T. spiralis (Larsh, 1967). These antigens have been shown to produce immunity, but serologic tests have failed to give evidence on the relative importance of humoral factors in accounting for this immunity.

The most convincing evidence for the direct actions of an antibody against T. spiralis has been provided by the study of precipitation antibodies by Oliver-Gonzalez (1941). When larvae or adult worms are incubated in antiserum,

protein precipitates occur around certain body openings. In view of the principles involved, there is no reason to doubt that these in vitro effects can also occur in vivo (Oliver-Gonzalez, 1940). Also, there is indirect evidence by Oliver-Gonzalez and Levine (1962) that precipitating antibodies play a role in inhibiting the normal development and reproductive potential of the worms during infection. Interference with metabolic activities could cause the stunting of worms in immunized hosts and also the reductions of numbers of muscle larvae that cannot be accounted for by a loss of adult worms from the small intestine.

Campbell (1955), and Chipman (1957) showed that soon after reinfection, these antibodies combine with antigens in areas of high concentration, and especially at the oral opening. This has been supported by the fluorescent-antibody studies of Jackson (1959). The source of "primary effective antigens" (excretions and secretions) appears to be the cells of the digestive tract, while the "secondary effective antigens" are probably secretions from the reproductive organs.

In considering humoral factors, Briggs and Deguisti (1963) showed the importance of those factors causing immediate hypersensitivity and the amount of immunity produced. In anaphylactic hypersensitivity, the reaction results from absorption of humoral antibody or antigen-antibody complexes onto susceptible mast cells in the presence of complement.

The cells are injured and they release amines, such as histamine and 5-hydroxytryptamine (serotonin), which are pharmacologically active agents.

The expulsion of the adult worms from the small intestine is due to factors associated with the characteristic intestinal inflammation. Therefore, it may be that the release of amines after a specific anaphylactic reaction in the mucosa causes sufficient tissue damage to initiate the inflammation. The fact that anti-histamines have an anti-inflammatory property (Campbell et al., 1963), and serotonin has a histamine-like action, supports this hypothesis.

The importance of trichinosis as a public health problem, plus the ease with which this parasite may be maintained in the laboratory, has led to its extensive laboratory use. Studies on the physiology and metabolism of the worm have contributed to the knowledge of parasitic processes, and a rational approach to chemotherapy. There is no satisfactory drug that can kill encapsulated T. spiralis larvae, or those larvae migrating through man's tissues without endangering him (Kagan, 1960). However, Fernando and Denham (1976) reported promising results using Mebendazole and Fenbendazole in mice.

Probably the two most important studies of trichinosis in terms of their clinical contributions to man were the Coker study (1956), in which it was demonstrated that cellular responses in mice were suppressed by the corticosteroid,

cortisone, and the Lord study (1958), which led to the discovery that ACTH was also effective. ACTH and cortisone reduced trauma in the host due to invasion of the larvae in the initial stages of infection, and alleviated the muscular pain, edema, and toxemic reactions associated with the infection.

A corticosteroid, prednisone, was used in this study to investigate the role of this agent in trichinosis. Prednisone ( $C_{21}H_{26}O_5$ ) is a synthetic derivative of cortisone, and is used widely in the treatment of a variety of diseases, including neoplastic, chronic inflammatory, or immunologic processes (Goodman, 1975). In spite of this wide usage, little is known about the mechanism of action of prednisone on the immune response. This drug produces several immunosuppressive effects, such as temporary lymphocytopenia, decreased immunoglobulin production, impaired delayed hypersensitivity reactions, activation or latent reactions, and enhanced susceptibility to infection (Targowski, 1975). The purpose of this study was to determine the effect of prednisone on immunoglobulin production and susceptibility to infection of mice with T. spiralis.

#### METHODS AND MATERIALS

Young female white mice, obtained from SASCO, Omaha, Nebraska, were used in this investigation when they were approximately eight weeks old. Eighty mice were divided

into four groups as follows: 1) prednisone-treated, T. spiralis-infected group containing thirty mice, 2) prednisone-treated group containing thirty mice, 3) T. spiralis-infected group with fifteen mice, and 4) control group (uninfected and prednisone-untreated) of five mice.

The mice were fed Purina brand rat chow, and water was available ad libitum. The animals were kept in stainless steel cages with sawdust bedding. Each cage contained no more than five mice.

The strain of Trichinella spiralis used in the study was obtained from a stock culture maintained at Drake University. The trichinella larvae were prepared for infection in the following manner. Two mice, infected with the stock parasite, were killed by cervical disjuncture and skinned. The feet, tail, and head were excised and discarded. The abdominal cavity was opened by a long incision so the diaphragm could be removed. A slide press, made from a diaphragm sample, was examined microscopically to confirm that the stock animals were adequately infected. The mice were then eviscerated leaving only the skeleton and musculature, which were then cut into small pieces.

An aqueous solution of 1% HCl (Fisher reagent ACS) and a 0.5% pepsin (Fisher NF) was prepared as the digest solution. The chopped up skeletal and muscular pieces of the two mice and 100 ml of the digest solution were blended for 30 seconds at 17,000 rpm in a Waring blender, according



to the method of Gallogly (1968). This blended mixture was then added to 300 ml of digest solution, and poured into a 500 ml screw top erlenmeyer flask. The digest mixture was incubated for eight hours at 37°C in a shaker (Eberback), during which time it was gently shaken.

After this eight hour period, the digest solution was allowed to settle to the bottom of the flask for fifteen minutes before 300 ml was aspirated off the top. Then 200 ml of lukewarm tap water was added to wash the mixture. This procedure was repeated three times, leaving 100 ml of the sample after the final wash.

Larvae were removed from the 100 ml worm suspension using a Pasteur pipette. The average number of larvae introduced into each animal to be infected was 43. This number was established by five trial counts of samplings of the worm suspension.

Prior to the inoculation of larvae, the mice were lightly anesthetized with ether (Merck USP). Then a Pasteur pipette containing the Trichinella larvae was eased down the esophagus of each mouse and the larvae suspension emptied into the stomach. Only the "prednisone-treated: T. spiralis-infected" and the "T. spiralis-infected" groups received the prepared larvae suspension. The other two groups, "prednisone-treated" and "control", were inoculated with tap water as a control.

Two of the experimental groups received the drug

prednisone: the "prednisone-treated: T. spiralis-infected" and the "prednisone-treated". The mice of these groups were given 1 ml injections intraperitoneally using a 2 mg/ml suspended solution of Orasone, (Rowell Laboratories), a commercial brand of prednisone.

The 2 mg/ml solution of Orasone was prepared for injection as follows. The Orasone, in 10 mg tablet form, was ground to a fine powder by means of mortar and pestle. Then, the powder was added to sufficient sterile distilled water and thoroughly mixed to arrive at the correct concentration. To facilitate solubility, four drops of 90% methanol was added to aid in the suspension of the prednisone.

The intraperitoneal injections of the prednisone solution were made using a tuberculin syringe and a 25 gauge needle. Beginning three days prior to infection with T. spiralis, and continuing throughout the study, 2 mg/ml of prednisone were given to the appropriate animal groups every other day.

The T. spiralis infection was followed for a 30 day period. (Prednisone injections, administered on alternate days, lasted a total of 33 days.) At the end of this period, serum samples were collected from 7 mice selected randomly from each experimental group, and from the 5 control mice. Blood samples, removed from the mice by means of cardiac puncture, were pooled for each group. The weights of the mice selected for blood samples were also recorded.

After removing the blood samples, the diaphragms of the mice infected with T. spiralis were removed. A transverse abdominal incision was made, and the diaphragm was exposed. A small incision was made into the periphery of the diaphragm so that iridectomy scissors and forceps could be used to excise the entire diaphragm from the rib cage. The diaphragm was then placed between two glass microscope slides and a press made so that the number of encapsulated larvae could be determined by microscopic examination.

The immune sera obtained from the experimental groups were analyzed using the agar gel-diffusion method and the precipitin test. The antigen used in both of these tests was Bacto-Trichinella Antigen (Difco Laboratories), a commercially prepared whole worm antigen which simulates the antigens produced by the organism when it is injected into the mice. By observing the precipitates formed in response to this antigen, and the antibodies present in the pooled serum samples, a qualitative estimate of the globin production by the mice was made.

Agar gel plates were made by dissolving 2 g of Noble agar (Difco) into 100 ml of water, and autoclaving this mixture. The sterile agar gel was then poured into sterile Petri dishes, and five wells cut with a sterile agar gel punch.

In Figure 1, the center well was filled with the commercial antigen by means of a capillary pipette. The

four surrounding wells were filled respectively with commercial antibody (Bacto-Trichinella Antibody, Difco Laboratories), the pooled sera samples from the "Trichinella-infected" group, the "prednisone-injected" group, and the "prednisone-injected: Trichinella-infected" group. The same arrangement was repeated with the commercial antigen diluted to the following concentrations: 1:2, 1:4, and 1:8. Replicate agar plates were made for each of the antigen dilutions, and incubated at 37°C and at 22°C. Once the antigen and antibodies were placed in their individual wells, the Petri dishes were wrapped in parafilm to help keep the agar moist and facilitate their diffusion towards each other.

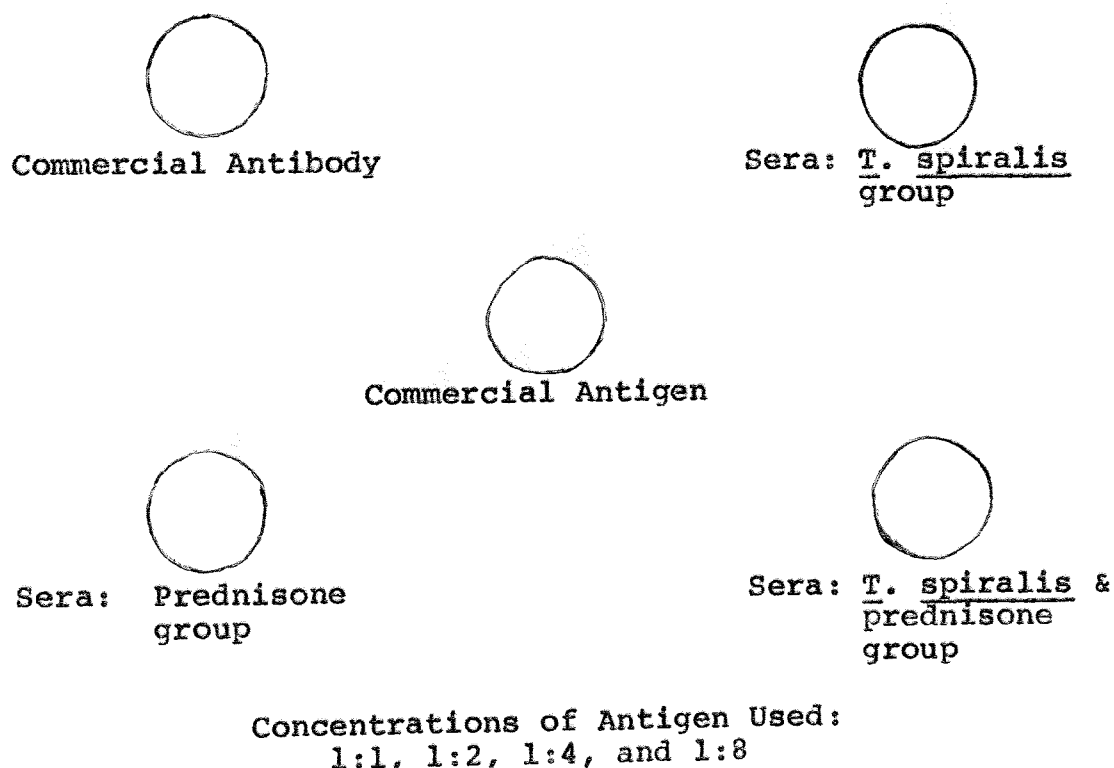


Figure 1

Diagram of Agar Gel Plates

The precipitin test was performed as an additional test for determining the presence of antibody in the sera from the experimental groups. A series of eight Durham tubes was prepared (Figure 2). The antigen and commercial antibody or sera samples were added to the individual tubes in equal amounts with Pasteur pipettes, being careful not to trap air bubbles at the interface. Separate commercial antigen and antibody controls were prepared against a 0.9% saline solution in tubes 1 and 2. With the exception of tube number 2, commercial antigen was added as the top layer in all tubes. Tube 3 contained the commercial antigen and the commercial antibody to test their activity.

The sera from the "prednisone-injected" group was used in tube 4 as an added control. The sera from the "Trichinella-infected" group was tested in tube 5 with undiluted commercial antigen, and with a 1:2 dilution of the antigen in tube 6. The sera from the "prednisone-injected: Trichinella-infected" group was tested in a like manner in tube 7 with undiluted antigen, and with a 1:2 dilution of the antigen in tube 8. The tubes were observed for the presence of a cloudy precipitin ring at the interface after 2 hours at room temperature, and then again after 24 hours of refrigeration at 4°C.

Tube 1Antigen  
Control

|             |
|-------------|
| Difco<br>Ag |
| Saline      |

Tube 2Antibody  
Control

|             |
|-------------|
| Saline      |
| Difco<br>Ab |

Tube 3Difco  
Ag-Ab

|             |
|-------------|
| Difco<br>Ag |
| Difco<br>Ab |

Tube 4Prednisone  
Group

|                     |
|---------------------|
| Difco<br>Ag         |
| Sera<br>From<br>(P) |

Tube 5Trichinella  
Group

|                     |
|---------------------|
| Difco<br>Ag         |
| Sera<br>From<br>(T) |

Tube 6Trichinella  
Group

|                     |
|---------------------|
| Difco<br>Ag<br>1:2  |
| Sera<br>From<br>(T) |

Tube 7Trichinella  
& Prednisone  
Group

|                       |
|-----------------------|
| Difco<br>Ag           |
| Sera<br>From<br>(T:P) |

Tube 8Trichinella  
& Prednisone  
Group

|                       |
|-----------------------|
| Difco<br>Ag<br>1:2    |
| Sera<br>From<br>(T:P) |

Ag. Antigen

Ab. Antibody

(P). Prednisone-injected group of mice

(T). Trichinella-infected group of mice(T:P). Trichinella-infected:prednisone-injected group of mice

Figure 2

Schematic of Precipitin Tests Used to Detect the Presence  
of Antibodies in Mice After Trichinella Infection and  
Alternate Day Prednisone Injections

## DATA AND DISCUSSION

Following infection with the Trichinella spiralis larvae, the groups of mice used in the study were checked daily. The mice developed normally except for some diarrhea during the first week post-infection. The Trichinella infected mice showed no apparent changes due to the infection, as substantiated by a normal weight gain and no loss of hair.

The prednisone injections given to the mice, however, incurred a high mortality rate. A pilot study to establish dosage levels was conducted early in the investigation, using 4 mg/ml of prednisone. This was administered on alternate days to a group receiving prednisone only, and to a group receiving both prednisone and T. spiralis. All mice receiving these injections died before 30 days (which was the time interval needed to complete the Trichinella life cycle). The prednisone dose was subsequently lowered to 2 mg/ml for the study. Fifteen of the 30 mice in the "prednisone-injected group survived the 33 day regimen of prednisone. In the "prednisone injected: Trichinella-infected" group, 24 of 30 mice survived.

The morality of the mice caused by the prednisone was perhaps due to toxicity, or its immuno-suppressive effects (Targowski, 1975), causing the mice to be more susceptible to environmental stresses.

The weights of all experimental mice were measured on day 33, following the Trichinella infection and prednisone

injection regimen, and are shown in Table 1. The control mice averaged 30.9 grams, the "Trichinella-infected" group 29.0 grams, the "prednisone-injected" group 29.0 grams, and the "Trichinella-infected:prednisone-injected" group averaged 27.6 grams.

Table 1. The Weights of Mice in Grams, After 33 Days Following Trichinella Infection and Alternate Day Prednisone Injections

| Mouse Number*      | Group   |                             |                     |  |
|--------------------|---------|-----------------------------|---------------------|--|
|                    | Control | <u>Trichinella</u> Infected | Prednisone Injected | <u>Trichinella</u> Infected: Prednisone Injected |
| 1                  | 31.3    | 30.5                        | 31.6                | 30.6   |
| 2                  | 30.7    | 23.6                        | 23.0                | 24.7   |
| 3                  | 32.1    | 32.3                        | 27.6                | 27.0   |
| 4                  | 32.8    | 27.0                        | 28.8                | 30.1   |
| 5                  | 27.9    | 29.3                        | 30.2                | 26.5   |
| 6                  | --      | 29.8                        | 30.6                | 28.4   |
| 7                  | --      | <u>31.1</u>                 | <u>31.0</u>         | <u>25.7</u>                                      |
| Mean               | 30.9    | 29.1                        | 29.0                | 27.6   |
| Standard Deviation | 1.89    | 2.93                        | 2.97                | 2.22   |

\*Mouse numbers apply separately to all groups.

At 33 days post-infection, the diaphragms of 10 randomly selected mice from the "Trichinella-infected" group, and the "Trichinella-infected:prednisone injected" group



were examined. The number of Trichinella larvae in the diaphragms of the mice of the two groups is shown in Table 2. The mean of the "Trichinella-infected" group was 640 Trichinella larvae per diaphragm with a standard deviation of 1.74. The "Trichinella-infected:prednisone-injected" group mean was 828 larvae per diaphragm with a standard deviation of 2.34. The mean of 828 Trichinella larvae in the "Trichinella-infected:prednisone-injected" group represents a 23% increase over the mean of 640 larvae in the "Trichinella-infected" group.

Table 2. Number of Trichinella Larvae in Whole Diaphragms of Mice After 33 Days Following Trichinella infection and Alternate Day Prednisone Injections

| Mouse<br>Number*      | Group  |                              |
|-----------------------|--|------------------------------|
|                       | <u>Trichinella</u> -Infected:<br>Prednisone-Injected | <u>Trichinella</u> -Infected |
| 1                     | 658  | 573                          |
| 2                     | 1152   | 965                          |
| 3                     | 734  | 623                          |
| 4                     | 768  | 847                          |
| 5                     | 864  | 654                          |
| 6                     | 1243   | 903                          |
| 7                     | 681  | 437                          |
| 8                     | 712  | 585                          |
| 9                     | 489  | 634                          |
| 10                    | 983  | 736                          |
| Mean                  | 828  | 640                          |
| Standard<br>Deviation | 2.34   | 1.74                         |

\*Mouse numbers apply separately to both groups.

It had been speculated that the prednisone would cause immunosuppression in the mice, and thereby lower their resistance to the Trichinella infection. In such a case, it might be assumed that more larvae would be deposited in the striated muscle, and that the diaphragm would also reflect this increase. This is shown in Table 2. Prednisone also inhibits synthesis of DNA, RNA, and proteins (Caron, 1967; Werthamer and Hicks, 1969), and decreases cellular metabolism by interfering with glucose uptake at the receptor sites in the cellular membranes (Claman, 1972). This activity may explain the lower average body weights in the groups treated with prednisone. Although the lowered average body weights of the experimental groups, as compared to the controls, can perhaps be partially attributed to stress from the Trichinella infection or the prednisone injection regimen; the difference in body weight strongly suggests that the prednisone acted in the predicted manner. Both the "Trichinella-infected" group and the "prednisone-injected" group had a lower average weight of 1.8 and 1.9 grams respectively, while the "Trichinella-infected:prednisone-injected" group had almost double this weight loss, 3.3 grams.

The presence of a protective factor in the serum of immunized animals was demonstrated by Mauss (1940), who found that T. spiralis larvae were significantly reduced in infectivity when incubated in antiserum. Oliver-Gonzalez (1940) reported that both larvae and adult worms died sooner (with

precipitates about their body openings) in antiserum than in normal serum. Hendricks (1950) showed an inverse relationship between the titer of specifically aroused circulating precipitins and the number of adult worms recovered after a challenging infection in mice given varying numbers of stimulating infections.

The specific nature of the anti-Trichinella antibody has been demonstrated frequently. This factor is not produced unless the host has been stimulated to produce it by infection with the parasite, or by inoculation with portions, extracts, secretions, or excretions of the parasite (Campbell, 1955). Antiserum transferred to non-immunized mice confers a small degree of passive immunity (Hendricks, 1953).

Coker (1956) has shown that an excess of cortisone leads to a decrease in lymphoid reserves and a decrease in the acquired immunity to Trichinella. Within this framework, the anti-Trichinella antibody operates normally, but its effectiveness in inducing an expression of immunity is compromised by cortisone's inhibitory effect on the cellular factors required to bring about the expulsion of the worm.

To determine the effect of prednisone on the antibody titer, agar gel-diffusion and precipitin tests were performed. The agar gel-diffusion plates did not show any reaction zones between the antigen and the serum samples. Especially noteworthy was the fact there was no reaction

between the commercially prepared antibody and antigen. Catty (1969) showed that after the 31st day of a primary Trichinella infection, the precipitin serum titers represent the maximum response to the antigens released in the intestine, but are still very low. Perhaps ultracentrifugation of larger sera samples could concentrate any serum precipitins present so that they could be detected by this test. However, since no reaction occurred between the commercial antiserum and antigen, the agar system itself could have contained an inhibitory factor preventing a precipitate from forming.

As an additional test of sera antibody levels, a precipitin test was performed, and the results are shown in Table III. Control tubes 1 and 2, with antigen and saline, and antibody and saline respectively, as expected, had no precipitin reaction. A precipitin ring was observed in tube 3 following 2 hours incubation at room temperature and following 24 hours of incubation at 4°C, confirming that the commercial antigen and antibody were reactive. Tube 4, using the sera from the "prednisone-injected" group, was negative as expected, since it is unlikely that the mice would have an innate antibody level to the Trichinella organism.

The precipitin test for tubes 5 and 6 ("Trichinella-infected" group) and for tubes 7 and 8 ("Trichinella-infected:prednisone-injected" group) showed no precipitate after 2 hours incubation at room temperature. Twenty-four

hours incubation at 4°C also failed to produce a precipitate. The sera used in the precipitin reactions from the experimental mice were recovered 33 days post-infection, but precipitin antibodies could not be detected in any of the tests. This was possibly because the level of antigenic stimulation used was too low, the number of induced infections was not adequate to produce a high level of precipitin antibodies, or the time at which the sera was drawn (immediately after the completion of the life cycle) was inappropriate for maximum antibody response.

Reaginic antibodies from Trichinella infections in the rat, rabbit, guinea pig, and man, possess a high specific activity and a high antigen-binding affinity (Bloch, 1967). There is, however, evidence that the serum of Trichinella infected mice appears to contain some Trichinella-specific antibodies which are non-precipitating with homologous antigens (Stoner and Hanks, 1960). No studies have been done with these antibodies using heterologous antigens.

The demonstration of reagins in Trichinella-infected hosts, even when these antibodies have been shown to be specifically reactive with the antigens of the infecting worm, does not prove that the reagins are a direct result of the infection. Demonstration of a definite role of these antibodies in reinfections must be done with a well-chosen system. It may be true that antibody production is closely associated also with the duration and site of antigenic

contact with the host. This may be especially true of gastro-intestinal infections where the local antibody production may be a key factor in the host's resistance.

Table 3. Precipitin Test Results Between Commercial Trichinella Antigen and Pooled Sera Samples From Mice After 33 Days Following Trichinella Infection and Alternate Day Prednisone Injections

| Tube Number | Description-Solutions Used                 | Antigen Dilution | Presence of Precipitin Ring:* |               |
|-------------|--|------------------|-------------------------------|---------------|
|             |  |                  | 2 Hours, Room Temp.           | 24 Hours, 4°C |
| 1           | Antigen Control                            | Undiluted        | -                             | -             |
| 2           | Antibody Control                           | Undiluted        | -                             | -             |
| 3           | Difco Antigen-Antibody                     | Undiluted        | +                             | +             |
| 4           | Prednisone Group Sera                      | Undiluted        | -                             | -             |
| 5           | <u>Trichinella</u> Group Sera              | Undiluted        | -                             | -             |
| 6           | <u>Trichinella</u> Group Sera              | 1:2              | -                             | -             |
| 7           | <u>Trichinella</u> : Prednisone Group Sera | Undiluted        | -                             | -             |
| 8           | <u>Trichinella</u> : Prednisone Group Sera | 1:2              | -                             | -             |

\*+ = presence of precipitin ring, - = absence of precipitin ring.

## CONCLUSION

The infectivity of Trichinella spiralis for the mice used in this study was established by diaphragm examination. The prednisone injections appeared to cause an immunosuppressive effect in the mice resulting in an increased infection with T. spiralis and a loss of body weight. Prednisone also seemed to prevent the development of an acquired immunity that normally develop 11 to 14 days post-infection, according to work done by Coker (1956). Coker suggested that the effects were probably due to an inhibition of the lymphoid-macrophage system and thus decreasing cellular immunity.

The mortality rate of the "prednisone-injected" group was higher than the "Trichinella-infected:prednisone-injected" group. Further study is needed to establish if the Trichinella parasite alters the prednisone effect as measured by mortality.

Antibodies in the sera of mice infected with Trichinella could not be demonstrated 33 days post-infection, utilizing the tube precipitin reaction and the agar gel-diffusion reaction with a commercial Trichinella antigen. It is likely that the antibody titer was below the minimal detection level for this time period. Other antibody detecting systems such as electrophoretic patterns or dermal sensitivity tests might show low antibody levels for animals used in this study.

Further studies into this problem are suggested by increasing the number of Trichinella parasites in the mice through repeated infections before the sera is collected in order to increase the antibody titer. Removal or further inhibition of antibody producing sites may also contribute to the understanding of the role of corticosteroid substances in altering the biology of the Trichinella parasite or the host.



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